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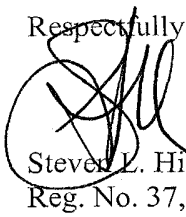
The examiner is invited to contact the undersigned attorney at 512-536-2487 with any questions relating to the referenced patent application.

Respectfully submitted,



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Date: August 27, 2001

APPENDIX A - MARKED COPY OF AMENDMENTS

In the Specification:

Please replace the paragraph at page 2, lines 3-8, with the following paragraph:

The present application is a division of U.S. Application Serial No. 09/244.675, filed February 4, 1999, and now issued as U.S. Patent No. 6,156,763, which claims the priority of [co-pending] U.S. Provisional Patent Application Serial No. 60/073,629, filed February 4, 1998[,]. [the] The entire disclosure of each of these applications [which] is incorporated herein by reference without disclaimer. The government may own rights in the present invention pursuant to contract number U19CA-67760-02, and contract number NCDDG, CA67760 from the National Cancer Institute, and contract number CA49751 and contract number CA77000 from the National Institutes of Health.

Please replace the paragraph at page 3, lines 4-13, with the following paragraph:

The ends of chromosomes have specialized sequences, termed telomeres, comprising tandem repeats of simple DNA sequences. Human telomeres consist of the sequence 5'-TTAGGG (SEQ ID No. 1) (Blackburn, 1991; Blackburn *et al.,* 1995). Telomeres have several functions apart from protecting the ends of chromosomes, the most important of which appear to be associated with senescence, replication, and the cell cycle clock (Counter *et al.,* 1992). Progressive rounds of cell division result in a shortening of the telomeres by some 50-200 nucleotides per round. Almost all tumor cells have shortened telomeres, which are maintained at a constant length (Allshire *et al.,* 1988; Harley *et al.,* 1990; Harley *et al.,* 1994) and are associated with chromosome instability and cell immortalization.

Please replace the paragraph at page 5, line 26 to page 6, line 11, with the following paragraph:

A preferred G-quadruplex structure is formed from the sequence d(AGGGTTAGGGTTAGGGTTAGGG) (SEQ ID No. 2) or the sequences d(TTAGGG)4 (SEQ ID No. 1), d(TAAGGGT)4 (SEQ ID No. 3), or d(TTAGGGTT)4 (SEQ ID No. 4) either alone or in the presence of a G-quadruplex interactive perylene diimide of general structure **I**. The structures were determined by NMR spectroscopy. Alternatively, one may determine the three-dimensional structure of potential G-quadruplex interactive agents by x-ray diffraction or molecular mechanics calculations. Preferred programs for determining the degree of complementarity between the potential G-quadruplex interactive agent and these G-quadruplex structures include DOCK, autoDOCK, AMBER and SYBYL. The preferred methods for generating orientations between the potential G-quadruplex interactive agents and these G-quadruplex structures are manual and using the DOCK or autoDOCK programs. The cutoff for determining the likelihood that the orientation of the potential G-quadruplex interactive agent and the G-quadruplex structure have sufficient chemical interaction to form a complex is roughly 75% of the favorable intermolecular interaction energy, calculated with the above programs, of the perylene diimide **2**–d(TTAGGG)4 (SEQ ID No. 1) complex structure as determined by NMR spectroscopy.

Please replace the paragraph at page 6, line 12 to page 7, line 10, with the following paragraph:

Preferred G-quadruplex structures are those formed by the sequences d(TTAGGG)4 (SEQ ID No. 1), d(AATGGGT)4 (SEQ ID No. 5) and d(TTAGGGTT)4 (SEQ ID No. 4). Several methods of determining the interaction of potential G-quadruplex interactive agents with these structures include UV/VIS spectroscopy, in which the changes in the UV/VIS spectrum of the potential agent under more than a 10% change at the wavelength due solely to the ligand and which undergoes the most change, upon addition of an excess of the G-quadruplex structure; UV spectroscopy, in which the melting temperature of the G-quadruplex structure as determined by a hyperchromicity transition at a given temperature range is increased by > 5°C upon addition of an excess of the agent; UV/VIS spectroscopy in which addition of a potential G-quadruplex interactive agent to a complex of a G-quadruplex-interactive perylene diimide and a G-quadruplex produces a >25% change in the absorption of due to the G-quadruplex-interactive

perylene diimine-G-quadruplex complex; UV/VIS spectroscopy in which addition of a potential G-quadruplex interactive agent to a complex of a G-quadruplex-interactive carbocyanine and a G-quadruplex produces a >25% change in the absorption of due to the G-quadruplex-interactive carbocyanine-G-quadruplex complex; NMR spectroscopy in which the melting temperature of the G-quadruplex as determined by the disappearance of the imino proton signals of the G-quadruplex is increase by >5°C in the presence of one- to two-equivalents of the agent; NMR spectroscopy in which the interaction of the agent with the G-quadruplex structure is determined by the shift of at least one of the imino protons of the G-quadruplex by >0.01 ppm upon addition of one- to two-equivalents of the agent; fluorescence spectroscopy in which the fluorescence emission spectrum of the agent undergoes a shift of > 5 nm and/or a change in intensity of >25% upon the addition of an excess of the G-quadruplex structure; fluorescence spectroscopy in which the fluorescence emission spectrum of a G-quadruplex-interactive perylene diimide–G-quadruplex complex undergoes a >25% change upon the addition of an excess of the agent; or fluorescence spectroscopy in which the fluorescence emission spectrum of a G-quadruplex-interactive carbocyanine–G-quadruplex complex undergoes a >25% change upon the addition of an excess of the agent.

Please replace the paragraph at page 11, lines 11-16 with the following paragraph:

FIG. 1. Effect of increasing concentrations of N,N'-bis(2-dimethylaminoethyl)-3,4,9,10-perylenetetracarboxylic acid diimide on inhibition of telomerase catalyzed extension of an 18-mer primer d[TTAGGG]$_3$ (SEQ ID No. 1) (1 μM). Elongated primer was labeled with 1.5 μM of [α-^{32}P]-dGTP (800 Ci mmol^{-1}, 10 mCi ml^{-1}) with 1 mM dATP and dTTP using a standard telomerase assay. Lanes 1-5 are 0, 10, 50, and 100 μM of N,N'-bis(2-dimethylaminoethyl)-3,4,9,10-perylenetetracarboxylic acid diimide.

Please replace the paragraph at page 11, lines 18-21 with the following paragraph:

FIG. 2. Changes in the UV/VIS absorbance spectrum of N,N'-bis(2-dimethylaminoethyl)-3,4,9,10-perylenetetracarboxylic acid diimide upon addition of increasing

amount of $[d(TTAGGGT)]_4$ (SEQ ID No. 6), an oligodeoxyribonucleotide which adopts a G-quadruplex structure.

Please replace the paragraph at page 11, lines 23-26 with the following paragraph:

FIG. 3. Titration of $[d(TTAGGG)]_4$ (SEQ ID No. 1) with N,N'-bis(2-piperdinoethyl)-3,4,9,10-perylenetetracarboxylic acid diimide.. Imino proton region of the 500-MHz 1H NMR is shown with increasing amounts of added ligand. The resonances labeled G4*, G5*, and G6* represent resonances of final 2:1 ligand/G-quadruplex complexes.

Please replace the paragraph at page 12, lines 1-3 with the following paragraph:

FIG. 4. NMR-based model of $[d(TTAGGG)]_4$ (SEQ ID No. 1)- N,N'-bis(2-piperdinoethyl)-3,4,9,10-perylenetetracarboxylic acid diimide complex. The ligand is stacked under the G6 guanine tetrad with positively charged side chains located in the grooves.

Please replace the paragraph at page 13, lines 13-23 with the following paragraph:

Telomerase is a ribonucleoprotein enzyme that synthesizes one strand of the telomeric DNA using as a template a sequence contained within the RNA component of the enzyme. The ends of chromosomes have specialized sequences, termed telomeres, comprising tandem repeats of simple DNA sequences which in humans is 5'-TTAGGG (SEQ ID No. 1) (Blackburn, 1991; Blackburn *et al.,* 1995). Apart from protecting ends of chromosomes telomeres have several other functions, the most important of which appear to be associated with replication, regulating the cell cycle clock and ageing (Counter *et al.,* 1992). Progressive rounds of cell division shorten telomeres by 50-200 nucleotides per round. Almost all tumor cells have shortened telomeres, which are maintained at a constant length (Allshire *et al.,* 1988; Harley *et al.,* 1990; Harley *et al.,* 1994) and are associated with chromosome instability and cell immortalization.

Please replace the paragraph at page 14, line 27 to page 15, line 2, with the following paragraph:

Human telomeres form structures known as G-quadruplexes. Human telomeres contain numerous repeats of the sequence TTAGGG (SEQ ID No. 1), exhibiting an enhancement of G and T residues and a paucity of A residues. Intramolecular G-quadruplex DNA may be designed by generating a sequence of human telomere repeats (FIG. 5). The G tetrad consists of four G bases hydrogen bonded in Hoogsteen fashion symmetrically disposed about a central axis, as shown in FIG. 5.

Please replace the paragraph at page 15, lines 3-19 with the following paragraph:

G-rich DNA is known to assume highly stable structures formed by Hoogsteen base pairs between guanine residues (Williamson, 1994; Nadel *et al.,* 1995). These structures, known as G-quadruplexes, are stabilized in the presence of K^+ and may have biological roles that are yet to be determined (Henderson *et al.,* 1987; Hardin *et al.,* 1997; Williamson *et al.,* 1989). One particular region of the genome where these structures may play a significant biological role is at the ends of chromosomes where G-rich DNA is normally found (*e.g.*, TTAGGG (SEQ ID No. 1) and TTGGGG (SEQ ID No. 7) tandem repeats in human cells and ciliate *Tetrahymena*, respectively) (Henderson *et al.,* 1987; Blackburn and Greider, 1995; Sundquist and Heaphy, 1993). In addition, a number of genes containing G-rich DNA have been identified recently, and it has been proposed that the G-rich regions within these genes may regulate gene expression by forming G-quadruplex structures (Sen and Gilbert, 1988; Hommond-Kosack *et al.,* 1993; Murchie and Lilley, 1992; Simonsson *et al.,* 1998). One potential biologically relevant role of G-quadruplex DNA is as a barrier to DNA synthesis (Howell *et al.,* 1996). This barrier has been thoroughly investigated and has been found to be K^+ dependent (Woodword *et al.,* 1994). This observation strongly suggests that the formation of G-quadruplex species is responsible for the observed effect on DNA synthesis (Weitzmann *et al.,* 1996).

Please replace the paragraph at page 15, lines 20-25 with the following paragraph:

The inventors have shown that the 2,6-diamidoanthraquinone BSU-1051 modulates human telomerase activity by a mechanism that is dependent on the elongation of the telomeric primer $d(TTAGGG)_3$ (SEQ ID No. 1) to a length that is then capable of forming an

intramolecular G-quadruplex structure (Sun *et al.*, 1997). The inventors have also shown that BSU-1051, by virtue of its interaction with G-quadruplex DNA, enhances the block of DNA synthesis by the G-quadruplex structure in the presence of K^+.

Please replace the paragraph at page 15, line 28 to page 16, line 8, with the following paragraph:

Several methods for identifying classes of G-quadruplex interactive agents may be employed. One method involves identifying compounds whose three-dimensional structure is complementary to that of the G-quadruplex structure. G-quadruplex structure is understood to mean at least in one sense the structure of the G-quadruplex that is formed by the single-stranded DNA corresponding to at least four repeats of the telomeric sequence. In humans, the telomeric sequence is d(TTAGGG) (SEQ ID No. 1). Thus, the G-quadruplex structure of interest for the identification of human telomerase inhibitors may be any sequence of the form {d([N_1]TTAGGG[N_2])}4 (SEQ ID No. 1) where [N_1] is zero to two bases corresponding to the human telomeric sequence; for example, [N_1] may equal G, GG, or may be absent; where [N_2] is zero to three bases corresponding to the human telomeric sequence; for example, [N_2] can equal T, TT, TTA or it may be absent.

Please replace the paragraph at page 16, lines 9-22, with the following paragraph:

Alternatively, G-quadruplex structure is understood to mean the fold-over or intramolecular G-quadruplex formed from at least four repeats of the G-triad of telomeric sequence. Thus, the G-quadruplex structure of interest for the identification of human telomerase inhibitors may be any sequence of the form d([N3][TTAGGG]3[N_2]) (SEQ ID No. 1) where [N_2] is as defined above and [N3] is three G's preceded by zero to three nucleotides corresponding to the human telomeric sequence. These structures may be determined by a variety of techniques including molecular mechanics calculations, molecular dynamics calculations, constrained molecular dynamics calculations in which the constraints are determined by NMR spectroscopy, distance geometry in which the distance matrix is partially determined by NMR spectroscopy, x-ray diffraction, or neutron diffraction techniques. In the

case of all these techniques, the structure can be determined in the presence or absence of any ligands known to interact with G-quadruplex structures, including but not limited to potassium and other metal ions, 2,6-diamidoanthraquinones, perylene diimides, or carbocyanines.

Please replace the paragraph at page 32, lines 10-17, with the following paragraph:

The relative inhibition of telomerase by N,N'-bis(2-dimethylaminoethyl)-3,4,9,10-perylenetetracarboxylic acid diimide was determined in a standard primer extension assay that does not use a PCR™-based amplification of the telomerase primer extension products. Briefly, the 18-mer telomeric primer d[TTAGGG]$_3$ (SEQ ID No. 1) (1 μM) without or with N,N'-bis(2-dimethylaminoethyl)-3,4,9,10-perylenetetracarboxylic acid diimide was elongated with telomerase in the presence of 1.5 μM of [α-^{32}P]-dGTP (800 Ci mmol^{-1}, 10 mCi ml^{-1}) with 1 mM dATP and 1 mM dTTP. The extension products were isolated and visualized by autoradiography after denaturing gel electrophoresis.

Please replace the paragraph at page 33, lines 5-13, with the following paragraph:

(A) A three dimensional structure of a candidate compound will be analyzed to determine their degree of complementarity to the three-dimensional structure of human telomeric DNA G-quadruplex. The NMR solution structure of d(AGGGTTAGGGTTAGGGTTAGGG) (SEQ ID No. 8) [pdb entry 143d] and its corresponding molecular surface, generated with the ms program, were used as inputs to the SPHGEN program. The resulting sphere cluster was used as input to DOCKv2.0 and a subset of the Cambridge crystallographic database was search using the contact scoring algorithm. N,N'-bis(2-dimethylaminoethyl)-3,4,9,10-perylenetetracarboxylic acid diimide was found to have one of the highest contact scores in the ~2000 compounds examined.

Please replace the paragraph at page 33, lines 14-21, with the following paragraph:

(B) Compounds may be selected for their ability to interact with human DNA G-quadruplex as indicated by UV/VIS spectroscopy. To a 10 μM solution of N,N'-bis(2-

dimethylaminoethyl)-3,4,9,10-perylenetetracarboxylic acid diimide in 20 mM phosphate buffer containing 100 mM KCl, pH 7.0 in a quartz cuvette was added 10 μL aliquots of a 3 mM solution of d(TTAGGGT)4 (SEQ ID No. 6). After each addition the UV/VIS spectrum was recorded. Pronounced changes in the UV/VIS spectrum of the compound were noted at wavelengths 488 nm (~40 % hypochromicity), 510 nm ~50 % hyperchromicity), and 548 nm (~200 % hyperchromicity).

Please replace the paragraph at page 33, lines 22-27, with the following paragraph:

(C) Compounds may be selected for their ability to interact with human DNA G-quadruplex as indicated by NMR spectroscopy. The imino proton spectrum (9-12 ppm) of a solution of d(TTAGGG)4 (SEQ ID No. 1) in D2O/H2O (10:90) was determined at 500 MHz. Aliquot of N,N'-bis(2-piperdinoethyl)-3,4,9,10-perylenetetracarboxylic acid diimide were added and the imino proton spectrum recorded. At an overall stoichiometry of 1:1 the G6 imino resonance becomes significantly broader and shifts >0.2 ppm upfield.

Please replace the paragraph at page 35, lines 9-23, with the following paragraph:

The assay is a modification of that described by Weitzmann, *et al.* Briefly, primers (24nM, sequence: 5'-TAATACGACTCACTATAG-3') (SEQ ID No. 9) labeled with [γ-^{32}P]ATP were mixed with template DNA PQ74(12nM,

sequence:TCCAACTATGTATACTTGGGGTTGGGGTTGGGG

TTGGGGTTGGGGTTAGCGGCACGCAATTGCTATAGTGAGTCGTATTA-3') (SEQ ID No. 10) in a Tris-HCl buffer (10mM Tris, pH8.0) containing 5mM K^+ and heated at 90°C for 4 min. After cooling at room temperature for 15 min. potential G-quadruplex-interactive compounds were then added to various concentrations. The primer extension reactions were initiated by adding dNTP (final concentration 100μM), $MgCl_2$ (final concentration 3 mM) and Taq polymerase (2.5 U/reaction, Boehringer Mannheim). The reactions were incubated at 55°C for 15 min. then stopped by adding an equal volume of stop buffer (95% formamide, 10mM EDTA, 10mM NaOH, 0.1% xylene cyanol. 0.1% bromophenol blue). The products were

separated on a 12% polyacrylamide sequencing gel. The gels were then dried and visualized on a phosphorimager (Molecular Dynamics model 445 S1).

Please replace the paragraph at page 36, lines 3-7, with the following paragraph:

The oligonucleotide G4A employed was synthesized on a Perseptive DNA synthesizer and deprotected following the routine phosporamidite procedures. the DNA was purified by polyacrylamide gel electrophoresis (PAGE). The sequence for this 39 oligomer single strand DNA is:

5′ CATGGTGGTTTGGGTTAGGGTTAGGGTTAGGGTTACCAC 3′ (SEQ ID No. 11).

Please replace the paragraph at page 36, line28 to page 37, line 18, with the following paragraph:

The N,N'-bis(3-morpholinopropyl)3,4,9,10-perylenetetracarboxilic acid diimide (KeTEL01) was synthesized from 3,4,9,10-perylenetetracarboxylic acid dianhydride and 3-morpholinopropylamine using a procedure analogous to that described above in example 5.3 for the synthesis of N,N'-bis(2-piperdinoethyl)-3,4,9,10-perylenetetracarboxylic acid diimide. A solution of KeTEL01 was prepared by dissolving 1 mg of KeTEL01 in 300 μL of 1 N HCl. To this solution was added 11 mL of a pH 7.0 buffer containing 20 mM sodium phosphate, 100 mM KCl, 1 mM EDTA, and 0.02% hydroxypropyl-β-cyclodextrin. Aliquots of this stock solution of KeTEL01 were transferred to 8 different quartz cuvettes and diluted into pH 7.0 mM sodium phosphate, 100 mM KCl, 1 mM EDTA buffer to afford solutions in which the concentration of KeTEL01 was 20 μM. To each of the cuvettes was added a solution of [d(TTAGGGT)]4 (SEQ ID No. 6) so that the final concentration of [d(TTAGGGT)]4 (SEQ ID No. 6) in each of the cuvettes was 0,4, 8, 12, 16, 20, 50, and 80 μM. These solutions were allowed to stand overnight in the dark, and the UV/VIS spectrum of each was determined. Pronounced, G-quadruplex concentration-dependent changes in the UV/VIS spectrum were noted at wavelengths 488 nm (~40% hypochromicity), 510 nm (~40% hyperchromicity) and 548 nm (~100% hyperchromicity). In a parallel study, changes in the UV/VIS spectrum of a 20 μM solution o f KeTEL01 in a pH 7.0 20 mM phosphate buffer containing 100 mM KCl and 1 mM EDTA were

determined upon the addition of 10 μM aliquots of a 3 mM (base pair) solution of calf thymus DNA. No changes in the UV/VIS spectrum of this solution were noted, indicating that KeTEL01 does not interact with double-stranded DNA.

Please replace the paragraph at page 37, lines 21-28, with the following paragraph:

A solution of 5 μM KeTEL01 in 20 mM phosphate buffer, 100 mM KCl, pH 7.0 was placed in a quartz cuvette and the UV/VIS spectrum determined. An aliquot of a solution of [d(TTAGGGT)]4 (SEQ ID No. 6) was added to the cuvette to afford a final concentration of 50 μM. The cuvette was quickly inverted several times and placed in the spectrophotometer. The absorption of the sample at 488nm was continuously monitored for 3 hours, during which time, the absorption decreased in a multiexponential function. The time required for the absorption at 488 nm to reach one-half of its equilibrium value was 60 min.

Please replace the paragraph at page 39, lines 15-18, with the following paragraph:

The DNA primer extension sequence P18 (5′-TAATACGACTCACTATAG-3′) (SEQ ID No. 12) and the template sequences shown in Table 1 were synthesized using a PerSeptive Biosystems Expedite 8909 synthesizer and purified with denaturing polyacrylamide gels. The template DNA was diluted to 5 ng/μL and dispensed into small aliquots.

Please replace the paragraph at page 40, line 26 to page 41, line 18, with the following paragraph:

To determine the nature of the G-quadruplex structures formed by the template sequences used in this study (see Table 1), dimethylsulfate (DMS) was used to probe the accessibility of N7 of guanine in the DNA templates (Maxam and Gilbert, 1980). When the PQ74 template was methylated in 1 × TE buffer, there was no apparent protection of any guanine N7. However, with the exception of the first guanine in each of the four TTGGGG (SEQ ID No. 7) repeats, all the guanines in the G-rich region of the PQ74 template are protected from reacting with DMS in 100 mM K^+ buffer, whereas guanines located outside the four repeats react strongly with DMS.

This DMS protection pattern for the G-rich region of the PQ74 template in K^+ buffer suggests that only three guanines in each of the four TTGGGG (SEQ ID No. 7) repeats are involved in G-tetrad formation. This DMS reaction pattern is different from that observed previously by Henderson and co-workers (Henderson *et al.,* 1990) with the $d(TTGGGG)_4$ (SEQ ID No. 7) G-quadruplex in which only the first guanine of the third repeat (corresponding to G9 in the PQ74 template) is hypersensitive to DMS methylation. On the basis of the results from the inventors' study, they propose a model for the G-quadruplex structure formed by the G-rich region of the PQ74 sequence consisting of $d(TTGGGG)_4$ (SEQ ID No. 7). In this model, the first guanine of the first repeat is located in the 5′ overhang region and is therefore open to DMS methylation. However, the first guanines of the second, third, and fourth repeats (G5, G9, and G13, respectively) are located in the loop regions of the G-quadruplex. Although the N7 groups of these three loop guanines are not involved in hydrogen bonds, steric inaccessibility may protect them from DMS methylation. The DMS footprinting pattern shows that while they are partially protected from DMS methylation, this protection is less than that for the other guanines in the repeat.

Please replace the paragraph at page 41, lines 19-24, with the following paragraph:

The TTAGGG (SEQ ID No. 1) repeats in the G-rich region of the HT-4 template also showed high DMS methylation protection in K^+ buffer. In this particular case, all three guanines in each repeat were almost evenly protected from methylation, indicating that all of them are involved in G-tetrad formation. This DMS methylation pattern is consistent with the intramolecular G-quadruplex structure proposed by Patel and co-workers for the $d[AG_3(T_2AG_3)_3]$ sequence based on NMR studies (Wang and Patel, 1993).

Please replace the paragraph at page 41, line 28 to page 42, line 27, with the following paragraph:

Although it has been shown that G-quadruplex structures block primer extension by DNA polymerase in a K^+ dependent manner (Weitzmann *et al.,* 1996), the inventors are unaware of any reports showing enhanced blockage by G-quadruplex—interactive agents. To determine if

BSU-1051 binding to G-quadruplex enhances the block to DNA synthesis, primer extension reactions were carried out in the absence and presence of BSU-1051. *Taq* DNA polymerase primer extension on DNA templates containing four repeats of either TTGGGG (SEQ ID No. 7) (PQ74) or TTAGGG (SEQ ID No. 1) (HT4) in the presence of different concentrations of BSU-1051 at 55°C were performed. In these studies, K^+ was added at low concentrations (5 mM of K^+ for the PQ74 template and 20 mM of K^+ for the HT4 template) in order to prevent overwhelming polymerase pausing due to formation of highly stable G-quadruplex structures. In the absence of BSU-1051, there is only a slight pausing of the *Taq* DNA polymerase when it reaches the 3′-end of the G-rich site on the template DNA at 55°C. However, upon increasing the concentration of BSU-1051, enhanced pausing is observed at the same site as that seen with low K^+ concentrations. This suggests that BSU-1051 enhances the polymerase pausing by stabilizing the G-quadruplex structure formed in the K^+ buffer. At high BSU-1051 concentrations, the inventors not only observed enhanced pausing at the 3′-end of the G-quadruplex site but also increased premature termination resulting from nonspecific interactions between BSU-1051 and the single-stranded template DNA. At a BSU-1051 concentration of 100 μM, the primer extension is completely inhibited due presumably to nonspecific interactions between BSU-1051 and the single- and/or double-stranded DNA or between BSU-1051 and the polymerase itself. In addition to the primary pausing site at the beginning of the G-quadruplex site, two other secondary pausing sites at the second and third G-rich repeats are observed at high BSU-1051 concentrations. These pausings are probably induced by other structures formed by this G-rich sequence. Given the fact that secondary pausing beyond the first G-tetrad is not seen in the sequencing lanes that contain 50 mM K^+, it is likely that these secondary pausings are caused by hairpin structures that are stabilized by BSU-1051 but not K^+. This suggests that BSU-1051 has a relatively higher affinity for G-quadruplex DNA over other DNA secondary structures or single- and double-stranded DNA.

Please replace the paragraph at page 43, lines 3-14, with the following paragraph:

To further evaluate the ability of BSU-1051 to stabilize G-quadruplex DNA, *Taq* DNA polymerase primer extension reactions were carried out at five different temperatures in the presence and absence of BSU-1051. In the absence of BSU-1051 polymerase pausing on the

PQ74 template containing four repeats of TTGGGG (SEQ ID No. 7) is almost lost at around 65°C, which is presumably the melting point of the G-quadruplex structure formed by this G-rich region in the template DNA. On the other hand, in the presence of 20 μM BSU-1051, the G-quadruplex structure is further stabilized, and significant pausing is observed up to 74°C. In the HT4 template containing four repeats of TTAGGG (SEQ ID No. 1) , in which the G-quadruplex structure formed is presumably less stable, pausing fades out at 55°C in the absence of the ligand. However, in the presence of BSU-1051, pausing is observed up to 65 °C. Thus, for both DNA sequences, ΔTm upon the addition of 20 μM BSU-1051 is about 20°C.

Please replace the paragraph at page 43, lines 15-30, with the following paragraph:

In order to confirm that the pausings seen result from the formation of a G-quadruplex structure on the template DNA, certain guanines in the templates were substituted with 7-deaza-dG. Since N7 of guanine is involved in hydrogen bonding in the formation of a G-quadruplex structure, substitution of guanine with 7-deaza-dG should preclude the formation of any G-quadruplex structure and allow for uninterrupted primer extension on the template by *Taq* DNA polymerase in the presence of either K^+ or BSU-1051. As shown in Table 1, two guanines in the TTAGGG (SEQ ID No. 1) repeat region of the HT4 template and four guanines in the TTGGGG (SEQ ID No. 7) repeat region of the PQ74 template were replaced with 7-deaza-dG. This change would allow the formation of no more than two intramolecular G-tetrads and should lead to destabilization of the intramolecular G-quadruplex structure. The primer extension results with these 7-deaza-dG substituted templates indicate that no significant pausing occurs in either template in the presence of up to 20 mM of K^+ or at BSU-1051 concentrations of up to 50 μM. This result provides strong support for the conclusion that BSU-1051 binds to and stabilizes intramolecular G-quadruplex DNA, leading to pronounced DNA synthesis arrest at the G-quadruplex site in the original G-rich templates.

Please replace the paragraph at page 44, lines 3-18, with the following paragraph:

G-rich sequences such as telomeric DNA and triplet DNA have been reported to form parallel or antiparallel G-quadruplex structures in the presence of monovalent cations such as

Na^+ and K^+. Williamson and co-workers observed very strong intramolecular UV cross-linking for the sequence $d(TTGGGG)_4$ (SEQ ID No. 7) in a 50 mM K^+ buffer (Williamson *et al.*, 1989). Their results indicate that this sequence forms an intramolecular structure. Using DMS methylation, the inventors conclude that four repeats of TTGGGG (SEQ ID No. 7) or TTAGGG (SEQ ID No. 1) within a non-G-rich sequence are capable of forming an intramolecular G-quadruplex structure in K^+ buffer. Furthermore, the DMS methylation results indicate that of the possible types of G-quadruplex structures that could be formed by $d(TTGGGG)_4$ (SEQ ID No. 7), a structure consisting of three G-tetrads is the predominant species in 100 mM of K^+ buffer. The proposed G-quadruplex structures formed by $d(TTGGGG)_4$ (SEQ ID No. 7) and $d(TTAGGG)_4$ (SEQ ID No. 7) repeats have diagonal loops, but alternative intramolecular G-quadruplex structures formed by foldover hairpins consisting of three G-tetrads are also possible (Williamson, 1994; Wang and Patel, 1995; Wang and Patel, 1994). However, the inventors could not differentiate between these two different types of intramolecular G-quadruplex structures by the DMS methylation pattern alone.

In the Claims:

Claims 1-24 and 26-36 have been canceled without prejudice or disclaimer.